PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PÓS-GRADUAÇÃO EM GERONTOLOGIA BIOMÉDICA
INSTITUTO DE GERIATRIA E GERONTOLOGIA

Ramón Hypolito Lima

UM ESTUDO SOBRE A PARTICIPAÇÃO DO CÓRTEX ENTORRINAL NA CONSOLIDAÇÃO E RECONSOLIDAÇÃO DA MEMÓRIA DE RECONHECIMENTO DE OBJETOS

Porto Alegre
2009

RAMÓN HYPOLITO LIMA

UM ESTUDO SOBRE A PARTICIPAÇÃO DO CÓRTEX ENTORRINAL NA CONSOLIDAÇÃO E RECONSOLIDAÇÃO DA MEMÓRIA DE RECONHECIMENTO DE OBJETOS

Dissertação apresentada ao Programa de Pós-Graduação em Gerontologia Biomédica do Instituto de Geriatria e Gerontologia da Pontifícia Universidade Católica do Rio Grande do Sul como requisito parcial à obtenção do grau de Mestre.

Orientador: Prof. Dr. Martín Cammarota

Porto Alegre

2009

RAMÓN HYPOLITO LIMA

UM ESTUDO SOBRE A PARTICIPAÇÃO DO CÓRTEX ENTORRINAL NA CONSOLIDAÇÃO E RECONSOLIDAÇÃO DA MEMÓRIA DE RECONHECIMENTO DE OBJETOS

Dissertação apresentada ao Programa de Pós-Graduação em Gerontologia Biomédica do Instituto de Geriatria e Gerontologia da Pontifícia Universidade Católica do Rio Grande do Sul como requisito parcial à obtenção do grau de Mestre.

Aprovada em____de_____ de 2009.

BANCA EXAMINADORA

_____

Prof. Dr. Irênio Gomes da Silva Filho

_____

Profa. Dra. Denise Cantarelli Machado

Porto Alegre

2009

Dedico esta dissertação a minha mãe Cecília Hypolito, que é o meu exemplo de integridade, garra e dedicação e que foi impreencindível para a conclusão desta etapa.

Muito obrigado por tudo que fizeste por mim.

Dados Internacionais de Catalogação na Publicação (CIP)

L732e Lima, Ramón Hypolito
Um Estudo sobre a participação do córtex entorrinal na consolidação e reconsolidação da memória de reconhecimento de objetos / Ramón Hypolito Lima. – Porto Alegre, 2009.
48 f.

Diss. (Mestrado) – Inst. de Geriatria e Gerontologia, PUCRS
Orientador: Prof. Dr. Martín Cammarota

1. Gerontologia Biomédica. 2. Memória. 3. Síntese de Proteína. 4. Reconhecimento de Objetos. 5. Córtex Entorrinal. I. Cammarota, Martín. II. Título.

CDD 618.97689

Bibliotecária Responsável: Salete Maria Sartori, CRB 10/1363

AGRADECIMENTOS

Em primeiro lugar, agradeço ao meu orientador **Martín**. Obrigado pelos ensinamentos, preocupação e carinho nestes anos de convivência. À **Lia** pelo carinho e pela honestidade nos conselhos.

Ao mestre, **Iván Izquierdo**, pela oportunidade de trabalhar sob seu comando desde o início de minha formação acadêmica. Obrigado por toda a atenção e por me mostrar que a ciência se faz com muito trabalho e dedicação.

Ao meu pai, **Mario Dimas** que mesmo distante soube me incentivar a continuar a busca por meus objetivos.

Ao meu irmão, **Daniel**, por estar sempre presente em todos os momentos da minha vida.

Aos demais familiares que de uma forma ou outra ajudaram, sendo peça fundamental na minha formação. Em especial à *mi abuela*, **Helsa Lima** por tudo que fez e ainda faz por mim e aos meus tios, de sangue e/ou de coração, **Dr. Álvaro Hypolito** e **Dr. Jarbas Vieira** que foram meu exemplo de dedicação a pesquisa. Agradeço muito por tudo.

Aos meus colegas e amigos de laboratório que foram importantíssimos para a conclusão de meus experimentos: **Janine**, **Jociane**, **Julia**, **Cristiane**, **Carolina**, **Siomara** e **Lucas**. Aos demais companheiros do Centro de Memória: **Natália**, **Gabriela**, **Juliana**, **Weber**, **Fernando**, **Cristiano**, **Pâmela**, **Clarice**, **Duda**,

Andressa, **Izadora** e **Larissa**. Obrigado ao Centro de Memória, a minha segunda casa.

A todos os meus amigos de longa data, por todo apoio e amizade nestes anos. Em especial: **Gabriel**, **Mateus**, **João Paulo**, **Julio**, **Érico**, **Guert**, **Marcel**, **Alexandre**, **Fernando**, **Tatiana** e **Milene**. A cada um, obrigado por fazerem parte de minha vida e a ter me ajudado a moldar a minha personalidade e caráter.

A todos os demais, não citados, que de alguma forma contribuíram para a elaboração desta dissertação.

Ao **CNPq** (Conselho Nacional de Desenvolvimento Científico e Tecnológico) pelo financiamento de minha bolsa nestes últimos dois anos.

Muito Obrigado,

Ramón Hypolito Lima

“I will study and get ready, and perhaps my chance will come”.

Abraham Lincoln

LISTA DE ABREVIATURAS

ANI	**Ani**somicina
CA1	**C**orno de **A**mon **1**, região do hipocampo, que os primeiros anatomistas julgaram ter formato semelhante ao chifre presente em algumas representações de Amon, rei dos deuses da cidade de Tebas, na mitologia do Antigo Egito
CA3	**C**orno de **A**mon **3**, região do hipocampo com nome de mesma origem da região citada anteriormente
CE	**C**órtex **E**ntorrinal
CHX	**C**iclo**h**e**x**emida
DA	**D**oença de **A**lzheimer
DMSO	Dimetil sulfóxido, Do inglês: **D**i**m**ethyl **s**ulf**o**xide
EME	**Eme**tina
ip	Injeção **I**ntra-**P**eritonial
MCD	**M**emória de **C**urta **D**uração
MLD	**M**emória de **L**onga **D**uração
MO	Estado Pertencente aos Estados Unidos da América, Do inglês: **M**iss**o**uri
RO	**R**econhecimento de **O**bjetos
USA	Estados Unidos da América, Do inglês: **U**nited **S**tates of **A**mérica.

RESUMO

Muitos estudos indicam que a formação e a persistência da memória de longa duração necessitam da síntese de novas proteínas em específicas áreas do cérebro. Neste trabalho demonstramos que a microinfusão de Anisomicina (ANI, 160 mg/ml), Emetina (EME, 50 mg/ml) e Ciclohexemida (CHX, 20mg/ml), inibidores de síntese protéica, quando administrados imediatamente, mas não 3h ou 6h após o treinamento na tarefa de reconhecimento de objetos prejudica a retenção da memória de longa duração, não afetando a memória de curta duração, assim como não afeta as atividades comportamentais. Quando ANI, EME e CHX são administradas no córtex entorrinal, logo após uma sessão de reativação envolvendo objetos iguais ou a combinação de um objeto familiar e um objeto novo, não afetam a persistência do processo de consolidação. Nossos dados sugerem que a síntese de proteínas no córtex entorrinal é necessária após o treinamento para a consolidação da memória de reconhecimento de objetos. Entretanto, a síntese de proteínas nesta região do cérebro não parece ser necessária para a reconsolidação da memória de reconhecimento de objetos.

ABSTRACT

Several studies indicate that formation and persistence of long-term memory entail the induction of protein synthesis in specific areas of the brain. Here we show that microinfusion of the protein synthesis inhibitors Anisomycin (ANI, 160 mg/ml), Emetine (EME, 50 mg/ml) and Cyclohexemide (CHX, 20mg/ml) in the entorhinal cortex immediately but not 3h or 6h after training rats in an object recognition learning task hinders long-term memory retention without affecting short-term memory or behavioral performance. When given into the entorhinal cortex after a memory reactivation session involving familiar objects or a combination of familiar and novel objects neither anisomycin nor emetine affected persistence of the consolidated trace. Our data suggest that protein synthesis in the entorhinal cortex is necessary early after training for consolidation of object recognition memory. However, reconsolidation of the recognition trace after retrieval does not seem to require protein synthesis in this area of the brain.

SUMÁRIO

I. APRESENTAÇÃO DO TEMA

No curso da evolução da vida na Terra, a capacidade de armazenar informações permitiu que os seres vivos se beneficiassem de experiências passadas para resolver problemas do dia a dia. Deste modo, tem sido observado que os grupos taxonomicamente mais antigos, como os invertebrados apresentam alguma capacidade mnemônica. No caso específico dos seres humanos a memória exerce um papel ainda mais nobre. Funciona como um arcabouço que armazena nossa história pessoal. Assim, é possível que mudemos com o passar dos anos[1].

Os termos aprendizado e memória possuem diferenças marcantes, embora muitas vezes sejam interpretados como sinônimos. Podemos definir aprendizado como um processo que produz uma alteração relativamente permanente no comportamento real ou potencial que ocorre como conseqüência da prática ou da experiência[2]. Já a memória seria a unidade informacional mais ou menos permanente, produto do processo denominado aprendizado. O conceito de memória inclui a aquisição, a formação, a conservação e a evocação de informações. Somente o que foi gravado pode ser lembrado e só é lembrado o que foi aprendido, ou seja, necessitamos aprender para gravar e gravar para evocar. Podemos dizer que a nossa memória *nos faz ser o que somos e aquilo que algum dia nós seremos*. Entretanto, a soma daquilo que esquecemos nos faz únicos[3].

Nem todas as memórias são iguais. Existem aquelas que perduram apenas o tempo suficiente para que possamos utilizá-las, mas também existem aquelas que persistem pelo resto de nossas vidas. Há memórias que dizem quem somos, de onde viemos e ajudam a predizer para onde vamos. Também formamos memórias que nos permitem realizar tarefas como, andar de bicicleta ou até escrever no computador este projeto. As memórias ainda podem ser classificadas de acordo com o seu tempo de duração (tabela 1) como memórias sensoriais, memórias de curta duração e memórias de longa duração.

Tabela 1: Classificação das memórias de acordo com o tempo que perduram.

	Tempo de permanência	***Características***
Memórias Sensoriais	Poucos segundos.	Retêm a breve impressão de um estímulo após este ter desaparecido, ou seja, depois que o sistema sensorial correspondente deixa de enviar informação ao cérebro.
Memórias de Curta Duração (MCD)	Menos de 3 horas.	Permite manter "na mente" e em um estado ativo e facilmente acessível, uma pequena quantidade de informação.
Memórias de Longa Duração (MLD)	Dias, meses, anos, a vida toda.	Contêm itens informacionais de diversa índole altamente interconectados entre si os quais se encontram armazenados de maneira mais ou menos permanente constituindo um sistema de arquivo dinâmico.

As memórias sensoriais são mantidas por alguns segundos e retêm a breve impressão de um estímulo após este haver desaparecido. As memórias de curta duração (MCD), também conhecidas como ativas ou primárias, são as memórias que perduram por algumas horas e armazenam uma pequena quantidade de informação. Já as MLD são aquelas que normalmente nos referimos quando coloquialmente falamos de "memória". Essas memórias armazenam uma grande quantidade de informações que apresentam, às vezes, uma alta complexidade de conexões. As MLD podem perdurar anos ou até mesmo a vida inteira[4].

As MLD também podem ser classificadas quanto ao seu conteúdo, sendo separadas em explícitas e implícitas (Tabela 2). As memórias explícitas contêm informações que usualmente sabemos que possuímos e as quais temos acesso consciente. Este tipo de memória inclui o conhecimento sobre nossa história pessoal e sobre o mundo que nos rodeia e podem ser divididas em duas subclasses: as memórias episódicas e as memórias semânticas[5,6].

Tabela 2: Classificação das memórias de longa duração de acordo com o conteúdo.

	Características	***Subdivisões e características***	
Explicitas (Declarativas)	Informações que usualmente sabemos que possuímos e a qual temos acesso consciente.	*Episódicas*	Guardam informação acerca de nossas próprias vidas e eventos.
		Semânticas	Armazenam informações acerca do mundo que nos rodeia, mas que lembramos sem saber como, quando e onde as adquirimos.
Implícitas (Não-declarativas)	Informações às quais não temos acesso consciente, tal como a informação obtida a partir de aprendizados simples como aqueles derivados pelo treino em tarefas de condicionamento clássico e habituação.	*Representação perceptual*	Representações (imagens, sons) sem significado aparente conhecido, mas úteis como dicas facilitatórias da evocação de informações inerentes; memória pré-consciente (priming).
		Procedimentos	Hábitos, habilidades, regras.
		Associativa	Associa dois ou mais estímulos (condicionamento clássico), ou um estímulo a uma resposta (condicionamento operante).
		Não associativa	Atenua uma resposta (habituação) ou a aumenta (sensibilização) através da repetição de um mesmo estímulo.

Quando recém adquiridas, as memórias são débeis e susceptíveis à ação de distintos tratamentos amnésicos. Porém, com o decorrer do tempo as memórias vão sendo progressivamente estabilizadas e fazendo-se resistentes aos agentes amnésicos através de um processo dependente de síntese de proteínas denominado consolidação[5,7,8]. A evocação pode tornar uma memória já consolidada novamente lábil à ação de distintos fármacos capazes de bloquear certos aspectos do metabolismo neuronal e de inibir a síntese protéica. Isto sugere a existência de um processo dependente da síntese de proteínas encarregado de re-estabilizar o traço que resultou debilitado como conseqüência de sua reativação[9,10,11,12,13].

Na década de 1950, Berlyne destacou que fatores como a novidade e a curiosidade são determinantes no comportamento exploratório de roedores[14]. Após alguns anos, foi proposto um paradigma para o estudo deste tipo de memória, a tarefa de Reconhecimento de Objetos (RO)[15]. Essa tarefa baseia-se na análise e observação de situações, eventos e/ou artefatos, familiares e

novos. Sabe-se que a consolidação da memória de RO requer o funcionamento normal da maquinaria celular encarregada da síntese de proteínas em distintas estruturas do lobo temporal. Além disso, experimentos recentes sugerem que a evocação da memória de RO torna o traço mnemônico novamente suscetível a intervenções farmacológicas tanto no córtex pré-frontal como no hipocampo[16,1718]. Pouco se sabe sobre a participação do Córtex Entorrinal (CE) em relação aos mecanismos existentes na evocação da memória de RO.

Anatomicamente o CE situa-se no lobo temporal do encéfalo, podendo haver pequenas variações em sua localização ao analisarmos grupos taxonomicamente distintos. Entre os roedores, o CE é localizado na região caudal do lobo temporal. Porém entre os primatas é situado no final da região rostral, possuindo extensões até a região dorsolateral do lobo temporal. O CE é dividido em região medial e região lateral, as quais são compostas por camadas. Essas camadas possuem distintas propriedades e conexões entre elas. A camada superficial II possui projeções para o giro dentado e para a região CA3 do hipocampo. A camada III possui projeções para a região CA1 e para o subículo. Uma característica importante do CE é a camada IV, também chamada de *lamina dissecans*, pois não possui corpo celular algum. As camadas mais profundas, especialmente a camada V, recebem aferências do hipocampo e de maneira recíproca conectam-se com outras regiões corticais[19,20,21].

O CE tem um papel chave na formação, evocação e extinção de memórias. Essa região cortical tem sido considerada parte fundamental nos circuitos que abrangem também o hipocampo, os núcleos amidalóides e muitas outras regiões do neocórtex em particular o córtex pré-frontal[22,23]. As evidências sobre o papel do CE em todas as formas de aprendizado relacionadas às patologias humanas surgiram a partir da análise do famoso paciente H.M.[24,25], até casos relativos a humanos com algum déficit cognitivo e/ou portadores da doença de Alzheimer (DA) em seus estágios iniciais[26].

A DA é caracterizada clinicamente por um déficit cognitivo progressivo e patologicamente por um declínio massivo de neurônios. Entretanto, um diagnóstico neuropatológico só é possível com análise histopatológica do tecido cerebral para a identificação de placas amilóides e dos emaranhados neurofibrilares[27]. Essas lesões são primeiramente encontradas no CE e

posteriormente no hipocampo atingindo posteriormente também outras regiões corticais. Lesões similares e com localização semelhante são observadas em indivíduos idosos, com ou sem déficit cognitivo aparente. Tem sido descrito que as placas amilóides e os emaranhados neurofibrilares são encontrados em pequenas quantidades em indivíduos normais a partir dos 20 anos de idade, aumentando de volume com o passar dos anos, porém nunca atingindo graus tão elevados como visto na DA[3,28,29,30,31]. Deste modo, alguns autores acreditam que o resultado da doença é conseqüência de um aumento do quadro histológico normal.

Tendo em vista a complexidade do processo mnemônico e as inúmeras estruturas neurais envolvidas neste processamento, é de grande importância estudos onde se investiguem a memória correlacionando o CE, a DA e o envelhecimento.

II. OBJETIVOS

II.1 Objetivo Geral

O objetivo do presente estudo foi verificar o requerimento de síntese de proteínas no CE durante a consolidação e reconsolidação da memória de RO.

II.2 Objetivos Específicos

- Verificar o requerimento de síntese de proteínas no CE durante o processo de consolidação do traço mnemônico, através da infusão bilateral de inibidores de síntese protéica, em distintos tempos após o treino na tarefa de RO.

- Estudar a necessidade de síntese protéica no CE durante o processo de reconsolidação do traço mnemônico, através da infusão bilateral de inibidores de síntese protéica, em diferentes tempos após a expressão da memória associada à tarefa de RO.

- Avaliar se a síntese protéica é requerida no CE para a formação de memórias de curta duração, através da infusão bilateral de inibidores de síntese de proteínas logo após o treinamento na tarefa de RO.

III. ARTIGO

Research article

Inhibition of protein synthesis in the entorhinal cortex blocks consolidation but not reconsolidation of object recognition memory

Ramón H. Lima[a, b], Janine I. Rossato[a], Cristiane Furini[a], Lia R. Bevilaqua[a], Iván Izquierdo[a, b], and Martín Cammarota[a, b].

[a.] *Centro de Memória, Instituto do Cérebro, Pontifícia Universidade Católica de Rio Grande do Sul, Av. Ipiranga 6690, RS 90610-000, Porto Alegre, Brasil.*

[b.] *Programa de Pós-Graduação em Gerontologia Biomédica, Pontifícia Universidade Católica de Rio Grande do Sul, Av. Ipiranga 6690, RS 90610-000, Porto Alegre, Brasil.*

Address correspondence to: Martín Cammarota, Centro de Memória, Instituto do Cérebro, Pontifícia Universidade Católica de Rio Grande do Sul (PUCRS), Porto Alegre, RS 90610-000. Email: mcammaro@terra.com.br or martin.cammarota@pucrs.br.

Keywords: object recognition, consolidation, reconsolidation, entorhinal cortex, anisomycin, emetine, cycloheximide, protein synthesis.

Acknowledgement: This work was supported by grants from the National Research Council of Brazil (CNPq).

Abstract

Memory consolidation and reconsolidation require the induction of protein synthesis in some areas of the brain. Here, we show that infusion of the protein synthesis inhibitors anisomycin, emetine and cycloheximide in the entorhinal cortex immediately but not 180 min or 360 min after training in an object recognition learning task hinders long-term memory retention without affecting short-term memory or behavioral performance. Inhibition of protein synthesis in the entorhinal cortex after memory reactivation involving either a combination of familiar and novel objects or two familiar objects does not affect retention. Our data suggest that protein synthesis in the entorhinal cortex is necessary early after training for consolidation of object recognition memory. However, inhibition of protein synthesis in this cortical region after memory retrieval does not seem to affect the stability of the recognition trace.

Introduction

Recognition memory allows to distinguish familiar from novel entities (Squire et al., 2007). Functional integrity of the medial temporal lobe is essential for encoding and expression of this type of information (Ennaceur & Delacour, 1988; Logothetis & Sheinberg, 1996; Clark, Zola & Squire, 2000; Riesenhuber & Poggio, 2002). Indeed, the anterograde amnesia observed in several patients with medial temporal lobe damage is characterized by the loss of recognition memory (Scoville & Millner, 1957). However, different areas of the medial temporal lobe seem to deal with different aspects of recognition memory processing (Balderas, Rodriguez-Ortiz, Salgado-Tonda, Chavez-Hurtado, McGaugh & Bermudez-Rattoni, 2008). Thus, while the hippocampus is essential for remembering contextual details and the temporal order of previous experiences, the perirhinal cortex appears to be mainly involved in familiarity detection (Brown & Aggleton, 2001; Rossato, Bevilaqua, Myskiw, Medina, Izquierdo & Cammarota, 2007; Myskiw, Rossato, Bevilaqua, Medina, Izquierdo & Cammarota, 2008; Suchan, Jokisch, Skotara & Daum, 2007).

The entorhinal cortex (EC) plays a crucial role in the communication between the hippocampus and sensory/association cortical areas. Indeed, the EC is the main source of projections to the hippocampus and also the primary output structure of the hippocampal formation (Canto, Wouterlood & Witter, 2008). The most prominent entorhinal output is directed to the perirhinal cortex which, in turn, regulates transmission of neocortical inputs to the EC (Pinto, Fuentes & Paré, 2006), suggesting that most hippocampal-cortical connections are controlled by a relay involving entorhinal-perirhinal interactions (Insausti et al., 1997). However, although inactivation of the EC impairs different types of hippocampus-dependent memories, including spatial, contextual and aversive learning (Ramirez et al., 1988; Ueki et al., 1994; Miwa & Ueki, 1996; Eijkenboom et al., 2000; Parron & Save, 2004a; Kopniczky et al., 2006; Bevilaqua et al., 2007), and it has been demonstrated that excitotoxic lesion of the EC impairs recognition (Parron & Save, 2004b; Mumby & Pinel, 1994; Galani, Weiss, Cassel & Kelche, 1998) little is known about the participation of the EC in object recognition (OR) memory. Considering that long-term memory (LTM) requires experience-dependent protein synthesis in areas of the brain relevant for information processing (Flexner, Flexner, Stellar, De La Haba &

Roberts, 1962; Flexner, Flexner, Roberts & De La Haba, 1965; Barondes & Cohen, 1967; Glassman et al., 1969; Squire & Barondes, 1972; Matthies et al., 1974; Flood, Bennett, Orme & Rosenzweig, 1975; Dunn & Leibmann, 1977; Davis & Squire, 1984; Luft, Buitrago, Ringer, Dichgans & Schulz, 2004; Gold et al., 2008; Rudy et al., 2008) we examined whether induction of protein synthesis is necessary in the entorhinal cortex for consolidation of OR LTM. Because evidence suggests that after retrieval OR LTM may briefly return to a fragile state and in order to persist must undergo a protein synthesis-dependent reconsolidation process (Kelly, Laroche & Davis, 2003; Bozon, Davis, & Laroche, 2003; Akirav & Maroun, 2006; Rossato et al., 2007, Maroun & Akirav, 2008) we also analyzed whether post-retrieval inhibition of protein synthesis in the EC affects OR memory retention.

Materials and Methods

Subjects, surgery and drug infusion: Naive male Wistar rats (3-month-old 280-300 g) raised in our own facilities or bought at FEPPS (Fundação Estadual de Produção e Pesquisa em Saúde do Rio Grande do Sul, Porto Alegre, Brazil) were used. The animals were housed 5 to a cage and kept with freely access to food and water under a 12/12 light/dark cycle (lights on at 7:00 AM). The animal's room temperature was maintained at 22-24^{o}C. Rats were bilaterally implanted with 27-gauge stainless steel cannulas into the entorhinal cortex under thiopental anesthesia (30-50 mg/kg). Coordinates were (in mm) 6.8 posterior to bregma, 5.0 lateral to the midline, and 8.1 ventral to the skull surface (Paxinos & Watson, 1986). Rats were given at least 4 days to recover before the experimental procedures. At the time of drug delivery, 30-gauge infusion cannulas were fitted into the guides. Infusions (1 μl/side) were carried out over 60 s using an infusion pump (KDS-200; kdScientific, USA). Placement of the cannulas was verified postmortem: 2-4 h after the last behavioral test, 1 μl of a 4% methylene-blue solution was infused as described above and the extension of the dye 30 min thereafter was taken as an indication of the presumable diffusion of the vehicle or drug previously injected. Only data from animals with correct implants were analyzed. All procedures were conducted in accordance with the "Principles of laboratory animal care" (NIH publication N°

85-23, revised 1996). Every effort was made to reduce the number of animals used and to minimize their suffering.

Drugs: Anisomycin (ANI), emetine (EME) and cycloheximide (CHX) were purchased from Sigma (St Louis, MO, USA). EME and CHX were dissolved in DMSO. ANI was dissolved in 1 M HCl, diluted in saline and the pH adjusted to pH 7.2-7.5 with NaOH. All drugs were stored in a light-proof container at -20^{o}C. Immediately before use, aliquots were thawed and diluted to working concentration with saline.

Object recognition task: The object recognition task was conducted in an open-field arena (60 x 40 x 50 cm) built of polyvinyl chloride plastic, plywood and transparent acrylic. Before training the animals were habituated to the experimental arena by allowing them to freely explore it 20 min per day for 4 days in the absence of stimulus objects. The stimulus objects were made of metal, glass or glazed ceramic. There were several copies of each object, which were used interchangeably. Glued to the base of each object was a rounded piece of Velcro, which was used to fix the objects to the arena's floor. The role (familiar or novel) and the relative position of the 2 stimulus objects were counterbalanced and randomly permuted for each experimental animal. All objects were behaviorally irrelevant and equally conspicuous for the rats as determined in pilot experiments and in previous reports (Rossato et al., 2007; Myskiw et al., 2008; Clarke, Rossato, Monteiro, Bevilaqua, Izquierdo & Cammarota, 2008). The open field arena and the stimulus objects were cleaned thoroughly between trials to ensure removal of olfactory cues. Exploration was defined as sniffing or touching the stimulus object with the nose and/or forepaws. Sitting on or turning around the objects was not considered exploratory behavior. A video camera was positioned over the arena and the rats' behavior was recorded using a video tracking and analysis system for later evaluation. The experiments were performed by an observer blind to the treatment condition of the animals. Data were expressed as percentage of the total exploration time in seconds.

Object recognition memory acquisition protocol: On day 1, rats were placed in the open field containing 2 different objects and left to freely explore them for 5 min. The test session was performed either 180 min (to analyze short-term memory; STM) or 24 hr after training (to evaluate LTM retention). In

the test sessions one of the objects was randomly exchanged for a novel object, and rats were reintroduced into the open field for 5 additional minutes.

Object recognition memory reactivation protocol: On day 1, rats were exposed to two different objects for 2 or 5 min. Twenty-four or 120 hours later, rats were re-exposed to the same two objects or exposed to of one the sample objects plus a new object for 2 or 5 min to reactivate the memory trace.

Results

To analyze whether protein synthesis in the EC is necessary for OR LTM, rats were trained in an object recognition task and, at different times after training, received bilateral intra-EC infusions of the protein synthesis inhibitor anisomycin (ANI; 160 µg/side; Rossato et al., 2007) or vehicle (VEH). Memory retention was evaluated 24 h posttraining. In the test session the animals were exposed for 5 min to one of the familiar objects presented during training together with a novel object. Rats that received VEH preferentially explored the novel object ($t_{(8)}$=4.57, p<0.005 in one-sample Student's t test with theoretical mean=50). Conversely, animals given ANI immediately (0 min) but not 180 min or 360 min after training spent the same amount of time exploring the two objects (Fig 1; $t_{(8)}$=3.80 and $t_{(8)}$=4.72, p<0.005 in one-sample Student's t test with theoretical mean=50 for ANI at 180 and 360 min posttraining, respectively).

It has been reported that, besides inhibiting protein synthesis, ANI may also disrupt other neural functions to interfere with memory formation (Canal, Chang & Gold, 2007). In order to rule out any ambiguous interpretation of our results, we analyzed the effect on OR LTM of two other widely used protein synthesis inhibitors, emetine (EME; Stollhoff, Menzel & Eisenhardt, 2008; 2005; Kraus, Schicknick, Wetzel, Ohl, Staak, Tischmeyer, 2002; Patterson, Alvarado, Rosenzweig & Bennett, 1988) and cycloheximide (CHX; Lai, Fan, Cherng, Chiang, Kao & Yu, 2008; Yu, Akalal & Davis, 2006; Duvarci, Nader, LeDoux, 2005; Pedreira, Pérez-Cuesta & Maldonado, 2004; Agin, Chichery, Maubert & Chichery, 2003). EME (50 µg/side) and CHX (20 µg/side) hampered OR LTM when infused in the EC immediately after training but had not effect when given in that cortex 180 min or 360 min posttraining (Fig 2; $t_{(8)}$=4.96, p<0.005 and $t_{(8)}$=2.80, p<0.05 for EME at 180 and 360 min posttraining, and $t_{(8)}$=3.18, p<0.05

and $t_{(8)}$=4.81, p<0.005 in one-sample Student's t test with theoretical mean=50 for CHX at 180 and 360 min posttraining).

When administered immediately after training, neither ANI nor EME or CHX affected OR STM as evaluated 180 min thereafter (Fig 3; $t_{(8)}$=3.19, p<0.05; $t_{(8)}$=4.72, p<0.005 and $t_{(8)}$=5.51, p<0.001 in one-sample Student's t test with theoretical mean=50 for ANI, EME and CHX, respectively).

To analyze whether inhibition of protein synthesis in the EC after retrieval affects persistence of the OR LTM trace, 24 h post-training rats were re-exposed for 5 min to the same objects presented during training and immediately after that received bilateral intra-EC infusions of ANI (160 µg/side). If protein synthesis in the EC were necessary for reconsolidation of recognition LTM then, when challenged with a familiar and a novel object one day after reactivation, the animals that received ANI after retrieval should not show preference for any object. However, as can be seen in Fig 4, when confronted with a familiar and a novel object on day 3, both VEH- and ANI-treated animals preferentially explored the novel one ($t_{(8)}$=3.97 and $t_{(8)}$=3.39 for the objects A and C; and $t_{(8)}$=9.18 and $t_{(8)}$=4.33 for the objects B and C in one-sample Student's t test with theoretical mean=50).

It has been suggested that the amnesia induced by some agents when given after retrieval may depend on the length of the memory reactivation session or the age of the mnemonic trace. However, post-retrieval intra-EC administration of ANI had no effect on OR LTM persistence when the trace was reactivated during 2 min instead of 5 min or when the reactivation session was carried out 120 h instead of 24 h after training (data not shown). We have previously demonstrated that retrieval in the presence of a novel object renders the reactivated recognition memory again susceptible to inhibition of hippocampal protein synthesis (Rossato et al., 2007). Nonetheless, as can be seen in Fig 5, ANI did not affect persistence of the OR LTM trace when given in the EC immediately after a reactivation session involving a familiar and a novel object.

Discussion

Our data show that posttraining intra-EC administration of ANI hinders OR LTM without affecting STM retention. This effect was time dependent and

mimicked by two other protein synthesis inhibitors, EME and CHX, suggesting that it was not due to a delayed detrimental action on perception, performance or on EC functionality and endorsing the hypothesis that consolidation of OR LTM requires protein synthesis in the EC during an early posttraining time window. This observation is important inasmuch it has been recently suggested that the amnesic effect of ANI could be due to a phenomenon different from protein synthesis inhibition (Canal et al., 2007; Radulovic & Tronson, 2008; Rudy, 2008).

The EC is usually perceived simply as a convergent point of cortico-hippocampal circuitry (Canto et al., 2008). Indeed, it was earlier proposed that the role of the EC during recognition would be restricted to maintaining the hippocampus in an "on" state to allow for the encoding of sensory information (Lee & Kesner, 2003; Nakazawa, Sun, Quirk, Rondi-Reig, Wilson & Tonegawa, 2003, Jensen & Lisman, 2005). However, our results suggest that the EC is not just a mere linking hub between the hippocampus and the neocortex but plays, instead, a key role in the consolidation of recognition memory in an enduring and useful form. Our findings are in line with others emphasizing the possible participation of the EC as a vital higher-order association center supporting LTM storage. Thus, reversible inactivation of the EC interferes with consolidation of aversive memory (Ferreira et al., 1992) and evidence indicates the EC takes part of a network supporting the lasting storage of non-spatial as well as spatial memories (Ross & Eichenbaum, 2006; Hebert & Dash, 2002). Moreover, it has been shown that grid cells in the EC are part of an environment-independent system that organizes positional, directional and translational information (Sargolini, Fyhn, Hafting, McNaughton, Witter et al., 2006) and operates in a way complementary to that of the hippocampus (Hebert & Dash, 2004)**.**

Unexpectedly, although the EC forms strong reciprocal connections with the perirhinal cortex (Pinto et al., 2006; Kerr, Agster, Furtak & Burwell, 2008) and relays polymodal sensory information to the hippocampus (Furtak, Wei, Agster & Burwell, 2007), two brain regions that play crucial roles in OR LTM reconsolidation (Akirav & Maroun, 2007; Rossato et al., 2007; Myskiw et al., 2008, Balderas et al., 2008), inhibition of protein synthesis in the EC after retrieval does not affect persistence of the recognition trace. This lack of effect is even more surprising since it has been reported that retrieval of OR memory

induces the phosphorylation of ERK1/2 in the EC (Kelly, Laroche & Davis, 2003), a step necessary for reconsolidation of different memory types in other brain regions (Duvarci, Nader & LeDoux, 2005; Miller & Marshall, 2005; Valjent, Corbillé, Bertran-Gonzalez, Hervé & Girault, 2006).

It is known that different factors, including the training-reactivation delay, the length of reactivation and the availability of relevant emotional or contextual cues at the moment of retrieval, determine the involvement of different brain areas in reconsolidation or even its occurrence (Pedreira & Maldonado, 2003; Cammarota et al., 2004; Suzuki et al., 2004; Power et al., 2006). In this respect, it has been shown that the effect of perirhinal cortex lesions on OR memory persistence depends on the number of exposures to the sample objects (Mumby, Piterkin, Lecluse & Lehmann, 2007) and Maroun and Akirav (2008) recently reported that presentation of an out-of context stressor impairs reconsolidation of OR memory in aroused but not in non-aroused rats. In the same way, we have previously shown that the hippocampus is engaged in OR memory reconsolidation only when new information is accrued to the original trace (Rossato et al., 2007). However, inhibition of EC protein synthesis after retrieval failed to affect persistence of the original OR memory regardless of the reactivation-test interval, the duration of the reactivation session or the introduction of a novel stimulus object during this session, suggesting that protein synthesis in the EC is not necessary for OR LTM reconsolidation, an observation that underlines the fact that, as for other memory types, reconsolidation of OR LTM is not simply the recapitulation of consolidation (Tronson & Taylor, 2007).

References

Agin, V., Chichery, R., Maubert, E. & Chichery, M.P. (2003). Time-dependent effects of cycloheximide on long-term memory in the cuttlefish. *Pharmacology, Biochemistry and Behaviour*. 1, 141-146.

Akirav, I. & Maroun, M. (2006). Ventromedial prefrontal cortex is obligatory for consolidation and reconsolidation of object recognition memory. *Cerebral Cortex*. 12, 1759-1765.

Akirav, I. & Maroun, M. (2007). The role of the medial prefrontal cortex-amygdala circuit in stress effects on the extinction of fear. *Neural Plasticity*. 30873.

Balderas, I., Rodriguez-Ortiz, C.J., Salgado-Tonda, P., Chavez-Hurtado, J., McGaugh, J.L. & Bermudez-Rattoni, F. (2008). The consolidation of object and context recognition memory involve different regions of the temporal lobe. *Learning and Memory*. 9, 618-624.

Barondes, S.H. & Cohen, H.D. (1967). Delayed and sustained effect of acetoxycycloheximide on memory in mice. *Proccedings of the National Academy of Science of the United States of America*. 58, 157-164.

Bevilaqua, L.R., Rossato, J.I., Clarke, J.H., Medina, J.H., Izquierdo, I. & Cammarota, M. (2007). Inhibition of c-Jun N-terminal kinase in the CA1 region of the dorsal hippocampus blocks extinction of inhibitory avoidance memory. *Behavioural Pharmacology*. 18, 483–489.

Bozon, B., Davis, S., & Laroche, S. (2003). A requirement for the immediate early gene zif268 in reconsolidation of recognition memory after retrieval. *Neuron*. 40, 695-701.

Brown, M.W. & Aggleton, J.P. (2001). Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nature Reviews. Neuroscience*. 1, 51-61.

Cammarota, M., Bevilaqua, L.R., Medina, J.H., Izquierdo, I. (2004). Retrieval does not induce reconsolidation of inhibitory avoidance memory. *Learning and Memory*. 11, 572-578.

Canal, C.E, Chang, Q. & Gold, P.E. (2007). Amnesia produced by altered release of neurotransmitters after intraamygdala injections of a protein synthesis inhibitor. *Proccedings of the National Academy of Science of the United States of America*. 104, 12500-12505.

Canto, C.B., Wouterlood, F.G. & Witter, M.P. (2008).What does the anatomical organization of the entorhinal cortex tell us? *Neural Plasticity*. 2008, 3812-3843.

Clark, R. E., Zola, S. M., & Squire, L. R. (2000). Impaired recognition memory in rats after damage to the hippocampus. *Journal of Neuroscience*, 20, 8853–8860.

Clarke, J.R., Rossato, J.I., Monteiro, S., Bevilaqua, L.R., Izquierdo, I. & Cammarota, M. (2008) Posttraining activation of CB1 cannabinoid receptors in the CA1 region of the dorsal hippocampus impairs object recognition long-term memory. Neurobiol Learn Mem. 90:374-81

Davis, H.P. & Squire, L.R. (1984). Protein synthesis and memory: a review. *Psychological Bulletin*. 96, 518-559.

Dunn, A.S. & Leibmann, S. (1977). The amnestic effect of protein synthesis inhibitors is not due to the inhibition of adrenal corticosteroidogenesis. *Behavioural Biology*. 19, 411-416.

Duvarci, S., Nader, K. & LeDoux, J.E. (2005). Activation of extracellular signal-regulated kinase- mitogen-activated protein kinase cascade in the amygdala is required for memory reconsolidation of auditory fear conditioning. *European Journal of Neuroscience*. 1, 283-89.

Eijkenboom, M., Blokland, A. & Van Der Staay, F.J. (2000). Modelling cognitive dysfunctions with bilateral injections of ibotenic acid into the rat entorhinal cortex. *Neuroscience*. 1, 27-39.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research*, 31, 47–59.

Ferreira, M.B., Wolfman, C., Walz, R., Da Silva, R.C., Zanatta, M.S., Medina, J.H. & Izquierdo, I. (1992). NMDA-receptor-dependent, muscimol-sensitive role of the entorhinal cortex in post-training memory processing. *Behavioural Pharmacology*. 3, 387-391.

Flexner, J.B., Flexner, L.B., Stellar, E., De La Haba, G. & Roberts, R.B. (1962). Inhibition of protein synthesis in brain and learning and memory following puromycin. *Journal of Neurochemistry*. 9, 595-605.

Flexner, L.B., Flexner, J.B., De La Haba, G., Roberts, R.B. (1965). Loss of memory as related to inhibition of cerebral protein synthesis. *Journal of Neurochemistry*. 7, 535-41.

Flood, J.F., Bennett, E.L., Orme, E. & Rosenzweig, M.R. (1975). Relation of memory formation to controlled amounts of brain protein synthesis. *Physiology & Behaviour*. 15, 97-102.

Furtak, S.C., Wei, S.M., Agster, K.L. & Burwell, R.D. (2007). Functional neuroanatomy of the parahippocampal region in the rat: the perirhinal and postrhinal cortices. *Hippocampus*. 17, 709-22.

Galani, R., Weiss, I., Cassel, J.C. & Kelche, C. (1998). Spatial memory, habituation, and reactions to spatial and nonspatial changes in rats with selective lesions of the hippocampus, the entorhinal cortex or the subiculum. *Behavioural Brain Research*. 96, 1-12.

Glassman, E. (1969). The biochemistry of learning: an evaluation of the role of RNA and protein. Annual Rev Biochem. 38, 605-646.

Gold, P.E. (2008). Protein synthesis and memory. Introduction. *Neurobiology of Learning and Memory*. 89, 199-200.

Hebert, A.E. & Dash, P.K. (2002). Extracellular signal-regulated kinase activity in the entorhinal cortex is necessary for long-term spatial memory. *Learning and Memory*. 9, 156-166.

Hebert, A.E. & Dash, P.K. (2004). Nonredundant roles for hippocampal and entorhinal cortical plasticity in spatial memory storage. *Pharmacology, Biochemistry and Behaviour*. 79, 143-153.

Insausti, A.M., Gazttelu, J.M., Gonzalo, L.M., Romero-Vives, M., Barrenechea, C., Felipo, V. & Insausti, R. (1997). Diet induced hyperammonemia decreases neuronal nuclear size in rat entorhinal cortex. *Neuroscience Letters*. 3, 179-181.

Jensen, O. & Lisman, J.E. (2005). Hippocampal sequence-encoding driven by a cortical multi-item working memory buffer. *Trends in Neurosciences*. 28, 67-72.
Kelly, A., Laroche, S. & Davis, S. (2003). Activation of mitogen-activated protein kinase/extracellular signal-regulated kinase in hippocampal circuitry is required for consolidation and reconsolidation of recognition memory. *Journal of Neuroscience*. 23, 5354-5360.

Kerr, K. M., Agster, K.L., Furtak, S.C. & Burwell, R.D. (2008). Functional neuroanatomy of the parahippocampal region: the lateral and medial entorhinal areas. *Hippocampus*. 17, 697-708.

Kopniczky, Z., Dochnal, R., Mácsai, M., Pál, A., Kiss, G., mihály, A. & Szabó, G. (2006). Alterations of behavior and spatial learning after unilateral entorhinal ablation of rats. *Life Science*. 78, 2683-2688.

Kraus, M., Schicknick, H., Wetzel, W., Ohl, F., Staak, S. & Tischmeyer, W. (2002). Memory consolidation for the discrimination of frequency-modulated tones in mongolian gerbils is sensitive to protein-synthesis inhibitors applied to the auditory cortex. *Learning and Memory*. 5, 293-303.

Lai, Y.T., Fan, H.Y., Cherng, C.G., Chiang, C.Y., Kao, G.S. & Yu, L. (2008). Activation of amygdaloid PKC pathway is necessary for conditioned cues-provoked cocaine memory performance. *Neurobiology of Learning and Memory*. 90, 164-170.

Lee, I. & Kesner, R.P. (2003). Differential roles of dorsal hippocampal subregions in spatial working memory with short versus intermediate delay. *Behavioural Neuroscience*. 117, 1044-1053.

Logothetis, N. K., & Scheinberg, D. L. (1996). Visual object recognition. *Annual Review of Neuroscience*, 19, 577–621.

Luft, A.R., Buitrago, M.M., Ringer, T., Dichgans, J. & Schulz, J.B. (2004). Motor skill learning depends on protein synthesis in motor cortex after training. *Journal of Neuroscience*. 24, 6515-6520.

Maroun, M. & Akirav, I. (2008). Arousal and stress effects on consolidation and reconsolidation of recognition memory. *Neuropsychopharmacology*. 33, 394-405.

Matthies, H. (1974). The biochemical basis of learning and memory. *Life Science*. 15, 2017-2031.

Miller, C.A. & Marshall, J.F. (2005). Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. *Neuron*. 47, 873-884.

Miskyw, J. C., Rossato, J. I., Bevilaqua, L. R., Medina, J. H., Izquierdo, I., & Cammarota, M. (2007). On the participation of mTOR in recognition memory. *Neurobiology of Learning and Memory*, 89, 338–351.

Miwa, C. & Ueki, A. (1996). Effects of entorhinal cortex lesion on learning behavior and on hippocampus in the rat. *Psychiatry and Clinical Neurosciences*. 4, 223-230.

Mumby, D.G. & Pinel, J.P. (1994). Rhinal cortex lesions and object recognition in rats. *Behavioural Neuroscience*. 108, 11-18.

Mumby, D.G., Piterkin, P., Lecluse, V. & Lehmann, H. (2007). Perirhinal cortex damage and anterograde object-recognition in rats after long retention intervals. *Behavioural Brain Research*. 185, 82-87.

Myskiw, J.C., Rossato, J.I., Bevilaqua, L.R., Medina, J.H., Izquierdo, I. & Cammarota, M. (2008). On the participation of mTOR in recognition memory. *Neurobiology of Learning and Memory*. 89, 338-351.

Nakazawa, K., Sun, L.D., Quirk, M.C., Rondi-Reig, L., Wilson, M.A. & Tonegawa, S. (2003). Hippocampal CA3 NMDA receptors are crucial for memory acquisition of one-time experience. *Neuron*. 38, 305-315.

Parron, C. & Save, E. (2004a). Evidence for entorhinal and parietal cortices involvement in path integration in the rat. *Experimental Brain Research*. 59, 349-359.

Parron, C. & Save, E. (2004b). Comparison of the effects of entorhinal and retrosplenial cortical lesions on habituation, reaction to spatial and non-spatial changes during object exploration in the rat. *Neurobiology of Learning and Memory*. 82, 1-11.

Patterson, T.A., Alvarado, M.C., Rosenzweig, M.R. & Bennett, E.L. (1988). Time courses of amnesia development in two areas of the chick forebrain. *Neurochemical Research*. 13, 643-647.

Paxinos, G., & Watson, C. (1986). The rat brain in stereotaxic coordinates. Academic Press: San Diego. pp. 119.

Pedreira, M.E. & Maldonado,H. (2003). Protein synthesis subserves reconsolidation or extinction depending on reminder duration. *Neuron*. 38, 863-869.

Pedreira, M.E., Pérez-Cuesta, L.M. & Maldonado, H. (2004). Mismatch between what is expected and what actually occurs triggers memory reconsolidation or extinction. *Learning and Memory*. 5, 579-585.

Pinto, A., Fuentes, C. & Pare, D. (2006). Feedforward inhibition regulates perirhinal transmission of neocortical inputs to the entorhinal cortex: ultrastructural study in guinea pigs. *The Journal of Comparative Neurology*. 6, 722-734.

Power, A.E., Berlau, D.J., McGaugh, J.L., Steward, O. (2006). Anisomycin infused into the hippocampus fails to block "reconsolidation" but impairs extinction: the role of re-exposure duration. *Learning and Memory*. 13, 27-34.

Radulovic, J. & Tronson, N.C. (2008). Protein synthesis inhibitors, gene superinduction and memory: too little or too much protein? Neurobiol *Learning and Memory*. 89, 212-218.

Ramírez, O.A., Orsingher, O.A. & Carrer, H.F. (1988). Differential threshold for long-term potentiation in the hippocampus of rats with inborn high or low learning capacity. *Neuroscience Letters*. 3, 275-279.

Riesenhuber, M., & Poggio, T. (2002). Neural mechanisms of object recognition. *Current Opinion in Neurobiology*, 12, 162–168.

Ross, R.S. & Eichenbaum H. (2006). Dynamics of hippocampal and cortical activation during consolidation of a nonspatial memory. *Journal of Neuroscience*. 26, 4852-4859.

Rossato, J. I., Bevilaqua, L. R., Myskiw, J. C., Medina, J. H., Izquierdo, I., & Cammarota, M. (2007). On the role of hippocampal protein synthesis in the consolidation and reconsolidation of object recognition memory. *Learning & Memory*, 14, 36–46.

Rudy, J.W. (2008). Is there a baby in the bathwater? Maybe: some methodological issues for the de novo protein synthesis hypothesis. *Neurobiology of Learning and Memory*. 2008 89, 219-224.

Sargolini, F., Fyhn, M., Hafting, T., McNaughton, B.L., Witter, M.P., Moser, M.B. & Moser, E.I. (2006). Conjunctive representation of position, direction, and velocity in entorhinal cortex. *Science*. 312, 758-762.

Scoville, W.B. & Millner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery and Psychiatry.* 20, 11–21.

Squire, L.R. & Barondes, S.H. (1972). Variable decay of memory and its recovery in cycloheximide-treated mice. *Proccedings National Academy Science of the United States of America*. 69, 1416-1420.

Squire, L.R., Wixted, J.T. & Clark, R.E. (2007). Recognition memory and the medial temporal lobe: a new perspective. *Nature Reviews. Neuroscience*. 11, 872-883.

Stollhoff, N., Menzel, R. & Eisenhardt, D. (2005). Spontaneous recovery from extinction depends on the reconsolidation of the acquisition memory in an appetitive learning paradigm in the honeybee (Apis mellifera). *Journal of Neuroscience*. 25, 4485-4492.

Stollhoff, N., Menzel, R. & Eisenhardt, D. (2008). One retrieval trial induces reconsolidation in an appetitive learning paradigm in honeybees (Apis mellifera). *Neurobiology of Learning and Memory*. 89, 419-425.

Suchan, B., Jokisch, D., Skotara, N. & Daum, I. (2007). Evaluation-related frontocentral negativity evoked by correct responses and errors. *Behavioural Brain Research*. 2, 206-212.

Suzuki, A., Josselyn, S.A., Frankland, P.W., Masushige, S., Silva, A.J., Kida, S. (2004). Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *Journal of Neuroscience*. 24, 4787-4795.

Tronson, N.C. & Taylor, J.R. (2007). Molecular mechanisms of memory reconsolidation. *Nature Reviews. Neuroscience*. 8, 262-275.

Ueki, A., Miwa, C., Miyoshi, K. (1994). Impairment in the acquisition of passive and active avoidance learning tasks due to bilateral entorhinal cortex lesions. *Journal of Neurological sciences*. 1, 14-21.

Valjent, E., Corbillé, A.G., Bertran-Gonzalez, J., Hervé, D. & Girault, J.A. (2006). Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. *Proccedings of the National Academy of Science of the United States of America*. 103, 2932-2937.

Yu, D., Akalal, D.B. & Davis, R.L. (2006). Drosophila alpha/beta mushroom body neurons form a branch-specific, long-term cellular memory trace after spaced olfactory conditioning. *Neuron.* 52, 845-855.

Figures

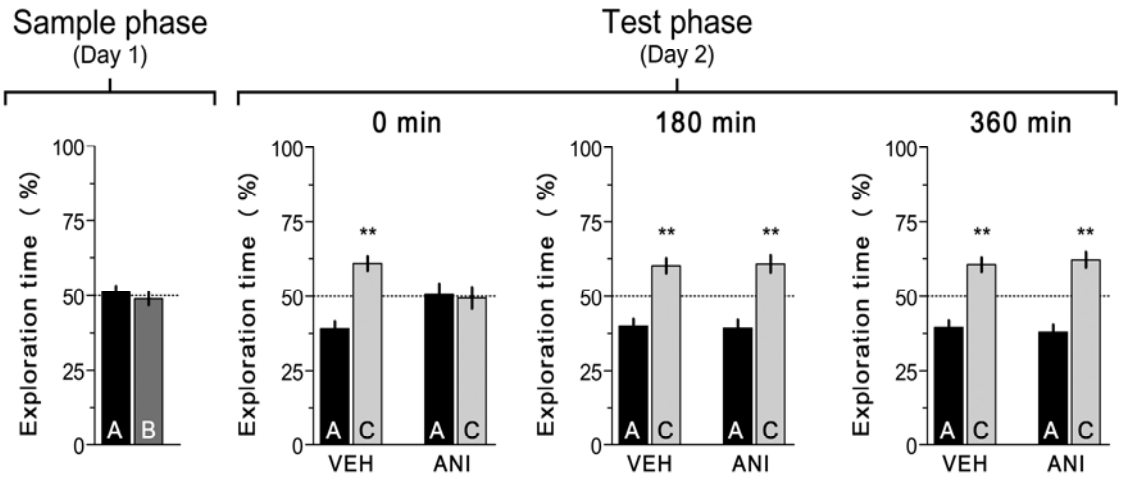

Fig 1: Post-training infusion of anisomycin in the entorhinal cortex blocks consolidation of object recognition long-term memory. On day 1 (Sample phase) rats (n=54) were exposed to 2 different objects (A and B) for 5 min and, at different times after that (0, 180 or 360 min), received bilateral infusions (1 µl/side) of vehicle (VEH) or anisomycin (ANI; 160 µg/side) in the entorhinal cortex. On day 2 (Test phase) the animals were exposed to a familiar (A) and a novel object (C) for 5 additional minutes to assess OR LTM retention. Data are presented as mean (± SEM) of the percentage of time exploring a particular object over the total time of object exploration. **p<0.005 in one-sample Student's t-test with theoretical mean=50; n=9 per group. Note that the animals that received ANI immediately after the sample phase spent the same amount of time exploring objects A and C during the test phase (Day 2; 0 min - ANI).

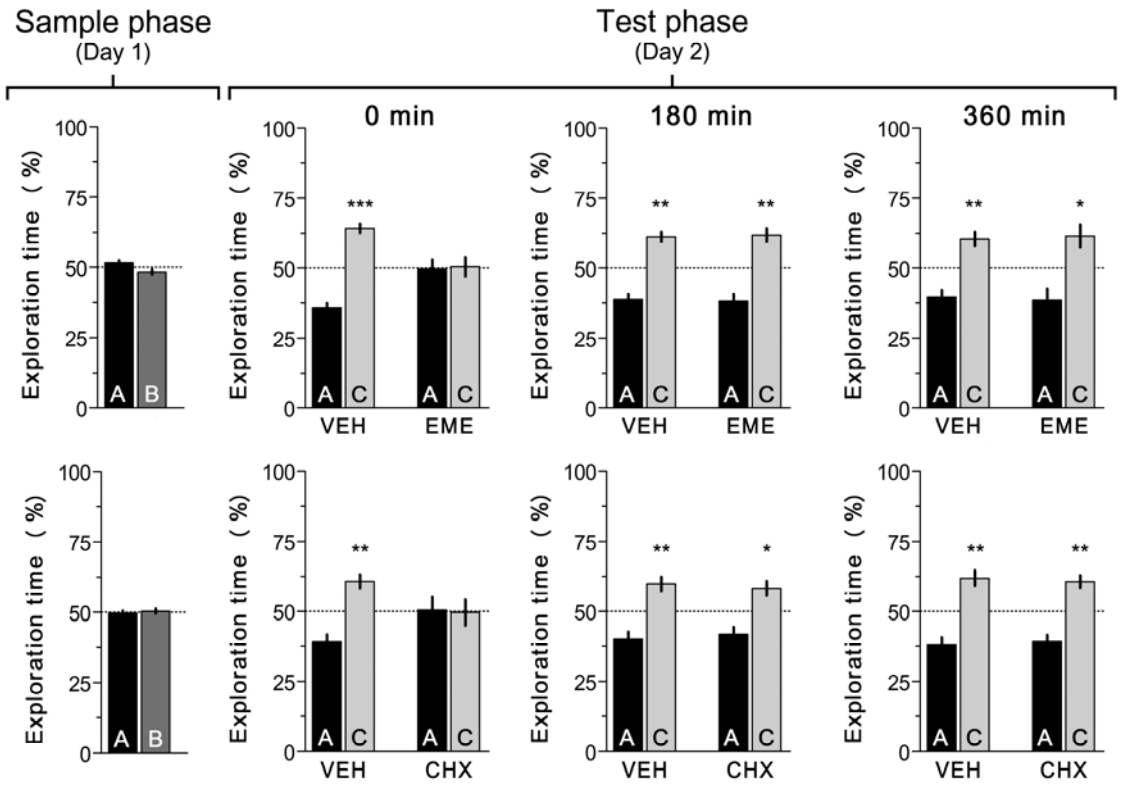

Fig 2: Post-training infusion of emetine and cycloheximide in the entorhinal cortex blocks consolidation of object recognition long-term memory. On day 1 (Sample phase) rats (n=108) were exposed to 2 different objects (A and B) for 5 min and, at different times after that (0, 180 or 360 min), received bilateral infusions (1 µl/side) of vehicle (VEH), emetine (EME; 50 µg/side) or cycloheximide (CHX; 20 µg/side) in the entorhinal cortex. On day 2 (Test phase) the animals were exposed to a familiar (A) and a novel object (C) for 5 additional minutes to assess OR LTM retention. Data are presented as mean (± SEM) of the percentage of time exploring a particular object over the total time of object exploration. ***p<0.001, **p<0.005 and *p<0.05 in one sample Student's t-test with theoretical mean=50; n=9 per group.

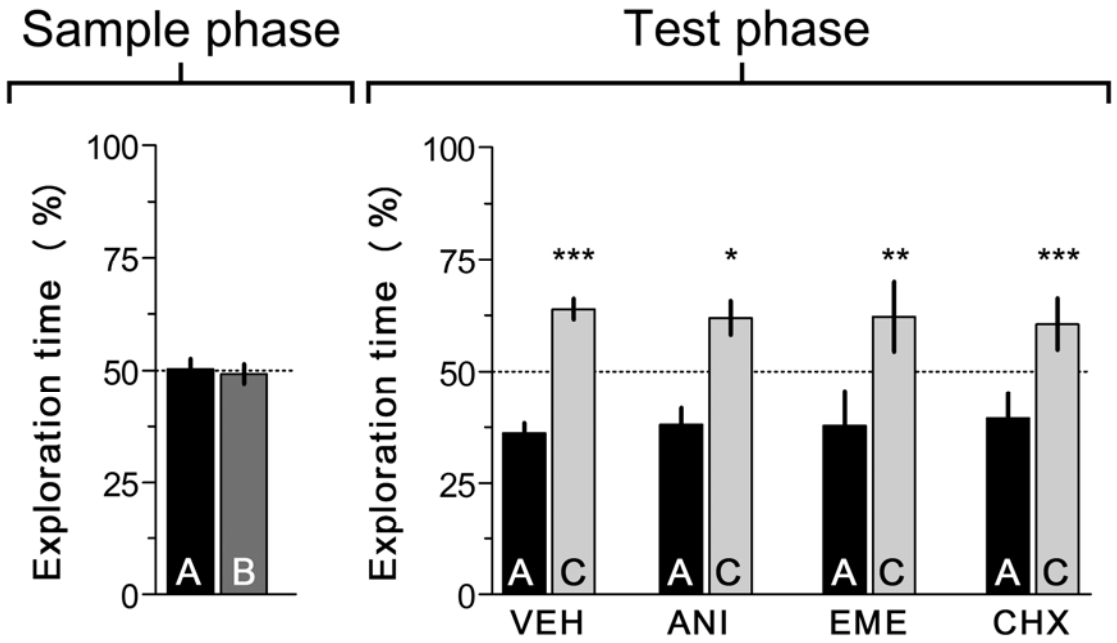

Fig 3: Posttraining inhibition of protein synthesis in the entorhinal cortex does not affect object recognition short-term memory. Rats (n=36) were exposed to 2 different objects (A and B) for 5 min (Sample phase) and immediately after that received bilateral infusions (1 µl/side) of vehicle (VEH), anisomycin (ANI; 160 µg/side), emetine (EME; 50 µg/side) or cycloheximide (CHX; 20 µg/side) in the entorhinal cortex. Three hours later (Test phase), animals were exposed to a familiar (A) and a novel object (C) for 5 additional minutes. Data are presented as mean (± SEM) of the percentage of time exploring a particular object over the total time of object exploration. ***p<0.001, **p<0.005 and *p<0.05 in one sample Student's t-test with theoretical mean=50; n=9 per group.

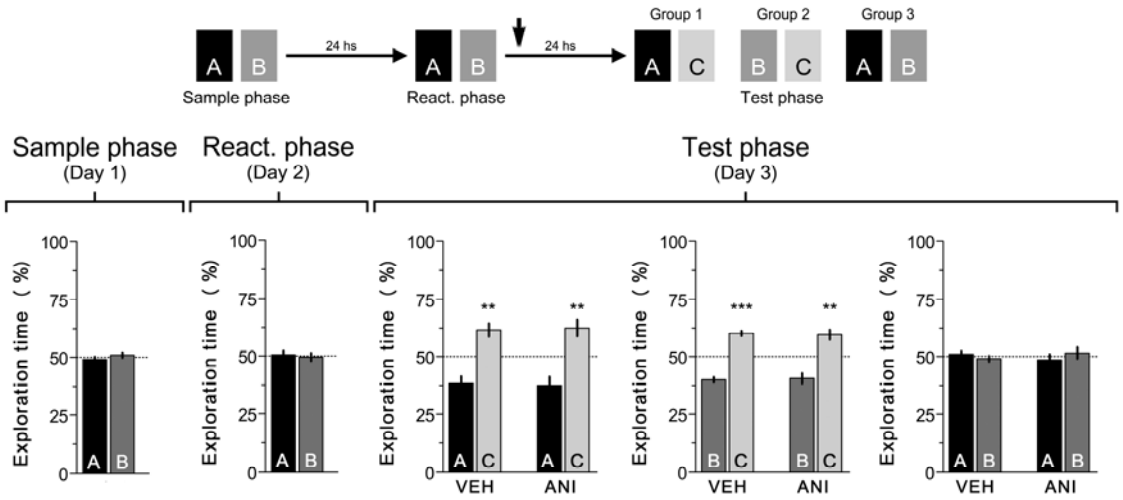

Fig 4: Inhibition of protein synthesis in the entorhinal cortex after a reactivation session involving exposure to familiar objects does not affect retention of object recognition long-term memory. Rats with infusion cannulas implanted in the entorhinal cortex (n=54) were exposed to two different objects (A and B) for 5 min (Sample phase, Day 1). Twenty-four hours later the animals were re-exposed for 5 additional minutes to the same two objects to reactivate the OR memory trace (Reactivation phase, Day 2) and immediately after that received bilateral infusions of vehicle (VEH) or anisomycin (160 µg/side; ANI) in the entorhinal cortex. Retention was assessed 24 h later by exposing the animals to the familiar objects A or B plus a novel object C (Test phase, Day 3). Note that the animals spent more time exploring the novel object C than the familiar objects A and B, indicating that memory had been preserved. ***p<0.001 and **p<0.005 one sample Student's t-test with theoretical mean=50; n=9 per group. The arrow-head indicates the moment of drug infusion.

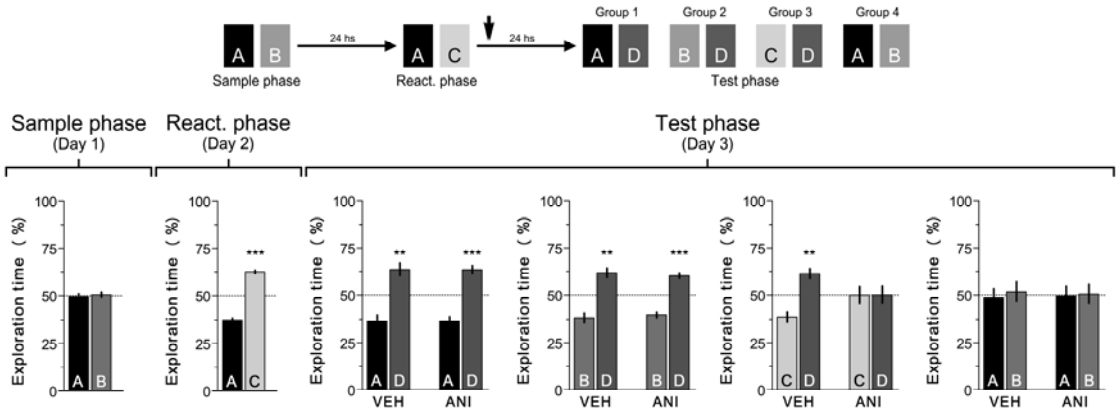

Fig 5: Inhibition of protein synthesis in the entorhinal cortex after a reactivation session involving exposure to a novel and a familiar object does not affect retention of object recognition long-term memory. Rats with infusion cannulas implanted in the entorhinal cortex (n=72) were exposed to two different objects (A and B) for 5 min (Sample phase; Day 1). Twenty-four hours later the animals were exposed to the familiar object A together with a novel object C (Reactivation phase; Day 2). Rats were randomly assigned to one out of four different groups and immediately after that received bilateral infusions of vehicle (VEH) or anisomycin (160 µg/side; ANI) in the entorhinal cortex. Twenty-four hours later the animals were submitted to a 5 min-long test phase (Test phase; Day 3) in the presence of different combinations of objects, as follows. Group 1= Object A + Object D; Group 2= Object B + Object D; Group 3= Object C + Object D, where D was a novel object, and Group 4= Object A + Object B. Note that ANI impaired retention of the memory for the novel object C first presented during the reactivation session but spared memory for familiar objects A and B. ***$p<0.001$ and **$p<0.005$ one sample Student's t-test with theoretical mean=50; n=9 per group. The arrow-head indicates the moment of drug infusion.

IV. CONSIDERAÇÕES FINAIS

O declínio cognitivo observado durante o envelhecimento é observável pela significativa perda da memória. Acredita-se que tais efeitos sejam causados principalmente por uma significativa redução na capacidade plástica das células neuronais[32].

Como previamente descrito, a DA leva a um agravamento observável por técnicas histológicas na morfologia, número de botões sinápticos e número de neurônios no SNC, em relação ao que ocorre nos processo fisiológico de envelhecimento[3,28,29,30,31]. Desta forma, a DA parece prejudicar a cognição de maneira semelhante ao envelhecimento normal, embora sua severidade e velocidade sejam infinitamente superiores (ver também para opiniões divergentes[33,34]).

O objetivo central desta dissertação foi verificar a necessidade de síntese de novas proteínas no CE durante os processos de consolidação e reconsolidação da memória de reconhecimento de objetos em ratos, um paradigma comportamental amplamente utilizado para o estudo neuroquímico e neurofarmacológico de memórias declarativas. As memórias declarativas são aquelas que armazenam informações às quais temos acesso consciente; inclusive àquelas pertinentes a fatos, eventos e objetos[6,35]. Nestas memórias baseia-se nosso conhecimento acerca da vida e do mundo, e são elas que definem a maneira como interagimos com outros indivíduos e com os diferentes componentes ambientais que nos rodeiam. Por serem determinantes das características da personalidade de cada indivíduo, a falha no seu processamento acarreta conseqüências devastadoras.

Este estudo possui relevância básica e clínica, buscando facilitar o entendimento das vias moleculares que envolvem a consolidação e a reconsolidação das memórias declarativas, possibilitando assim um desenvolvimento clínico no que diz respeito a novas terapias e tratamentos de doenças neurodegenerativas.

V. REFERÊNCIAS

1. Kandel ER, Schwartz JH, Jessel TM. Fundamentos da Neurociência e do Comportamento. Rio de Janeiro: Guanabara, 2000. 519 p.

2. Squire LR, Kandel ER. Memória: da mente às moléculas. Porto Alegre: Artmed, 2003. 251p.

3. Izquierdo I. Memória. Porto Alegre: Artmed, 2002. 92 p.

4. Izquierdo I, Cammarota M. Zif and the survival of memory. **Science**, 2004, 304 (5672)**:** 829–830.

5. Izquierdo I, McGaugh JL. Behavioural pharmacology and its contribution to the molecular basis of memory consolidation. **Behavioural Pharmacology**, 2000, 11 (7-8): 517-534.

6. Squire LR, Zola SM. (1996) Structure and function of declarative and nondeclarative memory systems. **Proc Nat Acad Sci USA**, 1996, 93:13515-13522.

7. McGaugh JL. Memory - a century of consolidation. **Science**, 2000, 287 (5451): 248-251.

8. Izquierdo I, Bevilaqua LR, Rossato JI, Bonini JS, Da Silva WC, Medina JH, Cammarota M. The connection between the hippocampal and the striatal memory systems of the brain: A review of recent findings. **Neurotoxicity Research**, 2006, 10: 113–121.

9. Judge ME, Quatermain D. Characteristics of retrograde amnesia following reactivation of memory in mice. **Physiology & Behavioural***,* 1982, 28 (4): 585-590.

10. Milekic MH, Alberini CM. Temporally graded requirement for protein synthesis following memory reactivation, **Neuron**, 2002, 24: 521–525.

11. Nader K, Schafe GE, Le Doux JE. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. **Nature**, 2000, 406(6797)**:** 722–726.

12. Przybyslawski J, Sara SJ. Reconsolidation of memory after its reactivation. **Behavioural Brain Research**, 1997, 84: 241–246.

13. Tronson NC, Taylor JR. Molecular mechanisms of memory reconsolidation. **Nature Reviews Neuroscience**, 2007, 8: 262–275.

14. Berlyne DE. Novelty and curiosity as determinants of exploratory behaviour. **British Journal of Psychology**, 1950, 41: 68-80.

15. Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. **Behavioural Brain Research**, 1988, 31 (1): 47-59.

16. Kelly A, Laroche S, Davis S. Activation of mitogen-activated protein kinase/extracellular signal-regulated kinase in hippocampal circuitry is required for consolidation and reconsolidation of recognition memory. **Journal Neurosci**, 2003, 23 (12): 5354-5360.

17. Akirav I, Maroun M. Ventromedial Prefrontal Cortex Is Obligatory for Consolidation and Reconsolidation of Object Recognition Memory. **Cerebral Córtex**, 2006, 16 (12): 1759-65.

18. Rossato JI, Bevilaqua RM, Myskiw JC, Medina JH, Izquierdo I, Cammarota M. On the role of hippocampal protein synthesis in the consolidation and reconsolidation of object recognition memory. **Neurobiology of Learning and Memory**, 2007, 14 (1): 36-46.

19. Hafting T, Fyhn M, Molden S, Moser M, Moser E. "Microstructure of a spatial map in the entorhinal cortex". **Nature**, 2005, 436 (7052): 801-6.

20. Hargreaves E, Rao G, Lee I, Knierim J. "Major dissociation between medial and lateral entorhinal input to dorsal hippocampus". **Science**, 2005, 308 (5729): 1792–4.

21. Fyhn M, Molden S, Witter M, Moser E, Moser M. "Spatial representation in the entorhinal cortex". **Science**, 2004, 305 (5688): 1258–64.

22. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. **Acta Neuropathol**, 1991, 82:239–259.

23. Gaomez-Isla T, Price JL, McKeel DJ, Morris JC, Growdon JH, Hyman BT. Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. **J Neurosci**, 1996, 16: 4491–4500.

24. Corkin S, Amaral DG, Gonzalez RG, Johnson KA, Hyman BT. H. M.'s medial temporal lobe lesion: findings from magnetic resonance imaging. **J Neurosci**, 1997, 17: 3964-3979.

25. Salat DH, van der Kouwe AJ, Tuch DS, Quinn BT, Fischl B, Dale AM, Corkin S. Neuroimaging H.M.: a 10-year follow-up examination. **Hippocampus**, 2006, 16: 936-945.

26. Braak H, Braak E. Neurofibrillary change confined to entorhinal region in Alzheimer disease. **Acta Neuropathol**, 1990, 80: 479-486.

27. Mizutani T. Neuropathological diagnosis of senile dementia of the Alzheimer type (SDAT): proposal of diagnostic criteria and report of the Japanese research meeting on neuropathological diagnosis of SDAT. **Neuropathology** 1994, 14: 91-103.

28. Braak H, Braak E. Staging of Alzheimer's disease-related neurofibrillary changes. **Neurobiol Aging**,1995, 16: 271-278.

29. Mizutani T, Kasahara M. Hippocampal atrophy secondary to entorhinal cortical degeneration in Alzheimer type dementia. **Neurosci Lett**, 1997, 222: 119-122.

30. Morrison JH, Hof PR. Life and death of neurons in the aging brain. **Science**, 1997, 278: 412–419.

31. Price JL, Ko AI, Wade MJ, Tsou SK, McKeel DW, Morris JC. Neuron

number in the entorhinal cortex and CA1 in preclinical Alzheimer disease. **Arch Neurol**, 2001, 58: 1395-1402.

32. Tapia-Arancibia L, Aliaga E, Silhol M, Arancibia S. New insights into brain BDNF function in normal aging and Alzheimer disease. **Brain Research Reviews**, 2008, 59: 201-220.

33. Gabrieli JDE. Memory systems analyses of mnemonic disorders in aging and age-related diseases. **Proc Natl Acad Sci USA**, 1996, 93: 13534-13540.

34. Buckner RL. Memory and Executive Function in Aging and AD: Multiple Factors that Cause Decline and Reserve Factors that Compensate. **Neuron**, 2004, 44: 195-208.

35. Squire LR, Zola-Morgan S. Memory: Brain systems and behaviour. **Trends Neurosci**, 1988, 11: 270-275.

ANEXOS

Aceite do comitê de ética

O projeto intitulado: **“Um estudo sobre a participação do córtex entorrinal na consolidação e reconsolidação da memória de reconhecimento de objetos”** foi aceito pelo comitê de ética para o uso de animais (CEUA) no dia 01 outubro de 2008, sob registro número 08/00036, no nome da coordenadora do CEUA, a Profa. Dr. Anamaria Feijó.

Artigos Publicados durante o mestrado

Izquierdo I, Bevilaqua LR, LIMA RH, Clarke JR, Costa JC, Cammarota M. Extinction learning: neurological features, therapeutic applications and the effect of aging. *Future Neurology*, v. 3, p. 133-140, 2008.

Bevilaqua LR, Rossato JI, Bonini JS, Myskiw JC, Clarke JR, Monteiro S, LIMA RH, Medina JH, Cammarota M, Izquierdo I. The role of the entorhinal cortex in extinction: influences of aging. *Journal of Neural Transplantation & Plasticity*, v. 2008, p. 1-8, 2008.

Cammarota M, Bevilaqua LR, Rossato JI, LIMA RH, Medina JH, Izquierdo I. Parallel memory processing by the CA1 region of the dorsal hippocampus and the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America (PNAS)*, v. 105, p. 10279-10284, 2008.

Izquierdo I, Bevilaqua LR, Rossato J I, LIMA RH, Medina JH, Cammarota M. Age-dependent and age-independent human memory persistence are enhanced by delayed post-training methylphenidate administration. *Proceedings of the National Academy of Sciences of the United States of America (PNAS)*, v. 08, p. 353-361, 2008.

LIMA RH, Rossato JI, Furini CR, Bevilaqua LR, Izquierdo I, Cammarota M. Infusion of Protein synthesis inhibitors in the entorhinal cortex blocks consolidation but not reconsolidation of object recognition memory. *Neurobiology of Learning and Memory*, v. xx, p. 466-72, May 2009.